Comparative Antimicrobial Spectrum and Activity of BMS284756 (T-3811; A Desfluoroquinolone) Tested Against Enteric Bacilli, Including *In Vitro* Test Comparisons and Development

A.M. Fix, M.A. Pfaller, D.J. Biedenbach, M.L. Beach, and R.N. Jones.
University of Iowa, Iowa City, Iowa, USA; and The JONES Group, North Liberty, Iowa, USA.

Ronald N. Jones, M.D.
The JONES Group / JMI Laboratories
345 Beaver Kreek Centre, Suite A
North Liberty, Iowa 52317
Phone: 319.665.3370
Fax: 319.665-3371
ronald-jones@jonesgr.com

ABSTRACT

Purpose: To determine the in vitro activity and MIC/disk test comparison of BMS284756 against isolates of Enterobacteriaceae from the SENTRY Antimicrobial Surveillance Program, 2000.

Methods: Isolates were tested using Etest and NCCLS reference broth and disk diffusion (5-µg disk) methods. In addition to BMS284756, ciprofloxacin, levofloxacin and gatifloxacin were tested by broth microdilution as comparator antimicrobial agents in the same class.

Results: 656 Enterobacteriaceae isolates (32 *Citrobacter* spp., 105 *Enterobacter* spp, 222 *E. coli*, 2 *H. alvei*, 145 *Klebsiella* spp., 14 *M. morganii*, 1 *P. agglomerans*, 57 *Proteus* spp., 12 *Providencia* spp., 9 *Salmonella* spp., and 57 *Serratia* spp.) were tested against BMS284756 and the comparator fluoroquinolones. BMS284756 was slightly less active than the other fluoroquinolones against these isolates (MIC_{90}, 4 µg/ml versus 0.06 - 2 µg/ml). However, at a proposed susceptible breakpoint of ≤ 4 µg/ml, 91% of isolates were susceptible to BMS284756, thus demonstrating a more equivalent spectrum of activity to the other quinolones. In general, isolates requiring >4 µg/ml of BMS284756 for inhibition of growth were less susceptible to each of the comparators as well suggesting cross-resistance is common among enteric pathogens. Excellent correlation was observed between broth dilution MICs and 5-µg disk zone diameters (r=0.94) and between broth dilution and Etest MICs (r=0.96).

Conclusions: BMS284756 demonstrated a spectrum of activity comparable to other fluoroquinolones against clinical isolates of Enterobacteriaceae. Both disk and Etest methods may be used to provide accurate susceptibility testing of BMS284756 against the Enterobacteriaceae through proposed susceptible breakpoint concentrations of ≤ 4 µg/ml. Higher breakpoints could require elevated disk concentrations of BMS284756.

INTRODUCTION

The increase of antimicrobial resistance has prompted the search for novel antimicrobial agents as well as more potent compounds within currently marketed drug classes. The quinolone class of antimicrobial agents has a wide spectrum of activity and has been used clinically with increasing frequency over the last three decades.

BMS284756, formerly T-3811, is a recent addition to the quinolone class which has comparable activity to older fluoroquinolones against enteric bacilli and enhanced activity against Gram-positive species including those that commonly cause community-acquired, typical and atypical pneumonia. BMS284756 is a novel des-fluoro(6)quinolone that differs from other quinolones in that it lacks fluorine at the C-6 position contributing to lower acute toxicity in mice. An oral dose of BMS284756 in mice has an area under the curve (AUC, µg h/ml) of 3.49 and a peak serum concentration of 1.9 mg/L making its bioavailability 96% versus ciprofloxacin which has an AUC of 0.90 and a peak serum concentration of 0.3 mg/L allowing a bioavailability of only 43%. Single dose (400 mg) pharmacokinetics in humans produced BMS284756 levels of 6.42 mg/L at 1.25 hours, a $T_{1/2}$ of 12.6 hours and an AUC of 84.1 µg x hr/mL.

This study was conducted to determine the in vitro activity of BMS284756 against enteric bacilli that commonly cause a wide variety of infections, and to establish preliminary disk (5-µg) diffusion breakpoint correlations. The evaluation of Etest (AB BIODISK, Solna, Sweden) compared to reference broth microdilution methodology results also determined MIC intermethod accuracy.

MATERIALS AND METHODS

A collection of recent Enterobacteriaceae clinical isolates from the 2000 SENTRY Antimicrobial Surveillance Program provided a total of 656 rapid growing isolates including *Citrobacter* spp. (32), *Enterobacter* spp. (105), *Escherichia coli* (222), *Hafnia alvei* (two), *Klebsiella* spp. (145), *Morganella morganii* (14), *Pantoea agglomerans* (one), *Proteus* spp. (57), *Providencia* spp. (12), *Salmonella* spp. (nine), and *Serratia* spp. (57). If identification was required, biochemical tests and VitekSystem (bioMerieux, Inc., Hazelwood, MO) were used to determine the species.

Isolates were tested by methods of the National Committee for Clinical Laboratory Standards (NCCLS) to determine broth microdilution MIC values and disk (5-µg) diffusion zone diameters. The disks were provided by BD Microbiology Systems (Cockeysville, MD, USA). In addition to these tests, Etest (AB BIODISK) MIC values using methods described in the manufacturer's product package insert were compared to the reference broth microdilution MIC results to determine intermethod agreement. All tests were incubated in ambient air at 35°C for 16-18 hours per NCCLS recommendations to determine the disk zone and MIC values. Ciprofloxacin, gatifloxacin, gemifloxacin, and levofloxacin were tested by broth microdilution to compare the potency of BMS284756 with antimicrobial agents within the same drug class. Results were compared by regression analysis using a proposed MIC susceptibility breakpoint of ≤ 4 mg/L determined by pharmacodynamic evaluations. All other interpretations of susceptibility (Table 1) were based on criteria of the NCCLS, except gemifloxacin where ≤ 0.25 µg/ml was used.

Quality control (QC) was achieved by testing American Type Culture Collection (ATCC) strains *E. coli* ATCC 35218 and 25922, *S. aureus* ATCC 29213 and 25923, *Enterococcus faecalis* ATCC 29212, and *Pseudomonas aeruginosa* ATCC 27853.

Figure 1: Scattergram comparing reference BMS284756 MIC results to the zone diameters of inhibition around 5-µg disks processed by NCCLS methods. Broken vertical line represents interpretive breakpoint suggested for Enterobacteriaceae (susceptible at ≥ 15 mm, resistant at ≤ 11 mm).

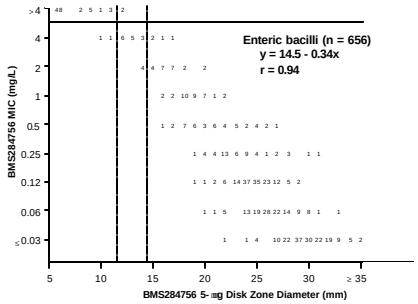

TABLE 1: Comparative activity of BMS284756 and four fluoroquinolone antimicrobial agents tested against 656 enteric bacilli isolates.

Organism (No. tested)	Antimicrobial Agent	MIC (mg/L) 50%	MIC (mg/L) 90%	% Susceptible[a]
Citrobacter spp. (32)	BMS284756	≤0.03	>4	87.5
	Ciprofloxacin	≤0.25	>2	87.5
	Gatifloxacin	≤0.03	>4	87.5
	Gemifloxacin	≤0.03	>4	87.5
	Levofloxacin	≤0.03	4	87.5
Enterobacter spp. (105)	BMS284756	0.12	4	92.4
	Ciprofloxacin	≤0.25	1	91.4
	Gatifloxacin	≤0.03	1	91.4
	Gemifloxacin	≤0.03	0.5	88.6
	Levofloxacin	≤0.03	2	91.4
E. coli (222)	BMS284756	≤0.03	0.25	90.1
	Ciprofloxacin	≤0.25	≤0.25	90.1
	Gatifloxacin	≤0.03	0.5	91.0
	Gemifloxacin	≤0.03	0.5	89.6
	Levofloxacin	≤0.03	0.5	91.0
Klebsiella spp. (145)	BMS284756	0.12	0.5	97.2
	Ciprofloxacin	≤0.25	≤0.25	95.9
	Gatifloxacin	≤0.03	0.25	97.9
	Gemifloxacin	≤0.03	0.06	93.1
	Levofloxacin	≤0.03	0.25	96.6
M. morganii (14)	BMS284756	0.5	>4	85.7
	Ciprofloxacin	≤0.25	>2	78.6
	Gatifloxacin	0.06	4	78.6
	Gemifloxacin	≤0.03	4	71.4
	Levofloxacin	≤0.03	>4	78.6
Proteus spp. (57)	BMS284756	0.5	>4	71.9
	Ciprofloxacin	≤0.25	>2	73.7
	Gatifloxacin	0.12	>4	77.2
	Gemifloxacin	0.06	>4	70.2
	Levofloxacin	0.06	>4	78.9
Providencia spp. (12)	BMS284756	4	>4	66.7
	Ciprofloxacin	2	>2	33.3
	Gatifloxacin	2	>4	66.7
	Gemifloxacin	4	>4	25.0
	Levofloxacin	2	>4	50.0
Serratia spp. (57)	BMS284756	1	4	98.2
	Ciprofloxacin	≤0.25	0.5	96.5
	Gatifloxacin	0.25	0.5	100.0
	Gemifloxacin	0.12	0.5	86.0
	Levofloxacin	0.12	0.5	98.2
Other[b] (12)	BMS284756	0.12	0.25	100.0
	Ciprofloxacin	≤0.25	≤0.25	100.0
	Gatifloxacin	≤0.03	0.12	100.0
	Gemifloxacin	≤0.03	0.06	91.7
	Levofloxacin	≤0.03	0.25	100.0

a. Susceptibility was based on NCCLS recommended breakpoint criteria and ≤ 0.25 mg/L for gemifloxacin and ≤ 4 mg/L for BMS284756.
b. Includes *Salmonella* spp. (nine strains), *H. alvei* (two strains) and *P. agglomerans* (one strain).

RESULTS

- The BMS284756 MIC_{90} results ranged from ≤ 0.03 to 4 mg/L, highest for *Providencia* spp. Only the MIC_{90} results for *E. coli* and *Klebsiella* spp. remained at ≤ 0.5 mg/L for BMS284756 (Table 1).
- Generally BMS284756 was less active than comparison agents as follows: versus ciprofloxacin (4- to 8-fold), versus gatifloxacin (2- to 4-fold), versus gemifloxacin (equal to ≥ 8-fold) and versus levofloxacin (2-fold).
- The spectrums of activity comparisons using published or proposed susceptible breakpoints shows greater similarity among quinolones when testing these representive enteric bacilli: gatifloxacin (91.5% susceptible) > levofloxacin (90.8%) = BMS284756 (90.7%) > ciprofloxacin (89.5%) > gemifloxacin (86.6%).
- Figure 1 shows the scattergram of BMS284756 reference MIC test values compared to the zone diameters around the 5-µg disk. Using a tentative susceptible breakpoint of ≥ 15 mm and the proposed pharmacokinetic-determined breakpoint of ≤ 4 mg/L for broth microdilution MIC, excellent correlation was achieved (r=0.94).
- There may be a need to use a greater disk concentration (≥ 10-µg) in order to achieve more accurate agreement between the disk diffusion results (zones) and reference MIC values above 4 mg/L.
- Etest MIC results (data not shown) were compared with broth microdilution MIC values showing that 63.4% were in complete agreement. Eliminating off-scales results (> or ≤ MIC endpoints), determined that 97.7% of the Etest MICs remained within ± one log_2 dilution, and 99.8% of results were within ± two log_2 dilutions.

CONCLUSIONS

- The alternative in vitro testing methods (disk diffusion, Etest) that were tested in this experiment may be used to provide accurate susceptibility testing results for BMS284756 against the Enterobacteriaceae.

SELECTED REFERENCES

1. Fung-Tomc J, Minassian B, Kolek B, Washo T, Huczko E, Bonner D. (2000b). In vitro antibacterial spectrum of a new broad-spectrum 8-methoxy fluoroquinolone, gatifloxacin. J Antimicrob Chemother 45:437-446.
2. Fung-Tomc JC, Minassian B, Kolek B, Huczko E, Aleksunes L, Stickle T, Tasho T, Gradelski E, Valera L, Bonner DP. (2000a). Antibacterial spectrum of a novel des-fluoro(6)quinolone, BMS284756. Antimicrob Agents Chemother 44:3351-3356.
3. Hayashi K, Todo Y, Hamamoto S, Ojima K, Yamada M, Kito T, Takahata M, Watanabe Y, Narita H. (1997). T-3811, a novel des-F(6)-quinolone: Synthesis and in vitro activity of 7-isoindolin-5-yl) derivatives, abstr. F158, p. 173. In: Program and abstracts of the 37th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, DC.
4. Takahata M, Mitsuyama J, Yamashiro Y, Yonezawa M, Araki H. Todo Y, Minami S, Watanabe Y, Narita H. (1999). In vitro and in vivo antimicrobial activities of T-3811ME, a novel des-F(6)-quinolone. Antimicrob Agents Chemother 43:1077-1084.
5. Takahata M, Mitsuyama J, Yamashiro Y, Araki H, Yamada H, Havakawa H, Todo Y, Minami S, Watanabe Y, Narita H. (1997). T-3811, a novel des-F(6)-quinolone: Study of pharmacokinetics in animals, abstr. F160, p. 173. In: Program and abstracts of the 37th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, DC.
6. Gajjar D, Grasela D, Bello A, Ge Z, Christopher L. (2000). Safety, tolerability, and pharmacokinetics of BMS284756, a novel des-F(6)-quinolone antibiotic, following single oral doses in health adult subjects, abstr. F2259, p. 36. In: Program and abstracts of the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, DC.
7. National Committee for Clinical Laboratory Standards (NCCLS). (2000). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Document M7-A5. Wayne, PA:NCCLS.
8. National Committee for Clinical Laboratory Standards (NCCLS). (2000). Performance standards for antimicrobial disk susceptibility tests, seventh edition. Approved standard, M2-A7. Wayne, PA:NCCLS.
9. National Committee for Clinical Laboratory Standards (NCCLS). (2001). MIC testing. Supplemental tables M100-S10 (M7). Wayne, PA:NCCLS.
10. National Committee for Clinical Laboratory Standards (NCCLS). (2001). Development of in vitro susceptibility testing criteria and quality control parameters. Approved guideline M23-A2. Wayne, PA:NCCLS.
11. Nagai A, Takahata M, Miyazaki M, Kawamura Y, Kodama T, Todo Y, Watanabe Y, Narita H. (1997). T-3811, a novel des-F(6)-quinolone: Toxicological evaluation, abstr. F162, p. 173. In: Program and abstracts of the 37th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, DC.